Cathepsin cysteine protease inhibitors and their use.

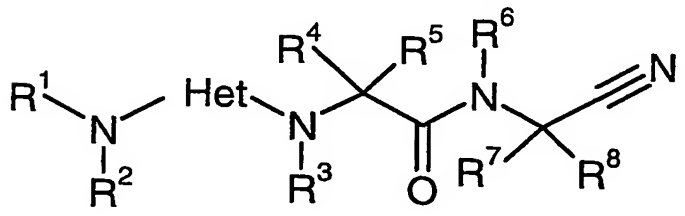
The present invention relates to compounds and compositions for treating diseases associated with cysteine protease activity. The compounds are reversible inhibitors of cysteine proteases S, K, F, L and B. Of particular interest are diseases associated with Cathepsin S. In addition this invention also discloses processes for the preparation of such inhibitors.

BACKGROUND OF THE INVENTION

Cathepsin S is a member of the papain superfamily of cysteine proteases which also encompasses Cathepsins B, H, L, O and K. Cathepsin S plays a key role in the processing of invariant chain in MHC class II complexes allowing the complex to associate with antigenic peptides. MHC class II complexes are then transported to the surface of the cell for presentation to effector cells such as T cells. The process of antigen presentation is a fundamental step in initiation of the immune response. In this respect inhibitors of cathepsin S could be useful agents in the treatment of inflammation and immune disorders such as, but not limited to, asthma, rheumatoid arthritis, multiple sclerosis and Crohn's disease. Cathepsin S has also been implicated in a variety of other diseases involving extracellular proteolysis such as the development of emphysema in COPD through degradation of elastin and in Alzheimers disease.

Other Cathepsins notably K and L have been shown to degrade bone collagen and other bone matrix proteins. Inhibitors of these cysteine proteases would be expected to be useful in the treatment of diseases involving bone resorption such as osteoporosis.

The present invention therefore provides a compound of formula (I)



(I)

R^1 is independently hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl

R^2 is independently aryl, heteroaryl or a group C_{1-6}alkylR^9, $CO(C_{1-6}$alkyl$)R^9$ or $SO_2(C_{1-6}$alkyl$)R^9$; where R^9 is aryl or heteroaryl

or R^1 and R^2 together with the nitrogen atom to which they are attached form a 4 to 7-membered saturated ring optionally containing a carbonyl group, O, S or N atom and optionally substituted by one or more C_{1-6} alkyl, amino, hydroxy, CO_2C_{1-6} alkyl, COC_{1-6} alkyl, halogen, C_{1-6} alkylhydroxy, $NR^{10}R^{11}$ where R^{10} and R^{11} are independently hydrogen, C_{1-6} alkyl or together with the nitrogen atom to which they are attached form a 5- or 6-membered saturated ring optionally containing a further O, S or NR^1 group, C_{1-6} alkyl$NR^{12}R^{13}$ where R^{12} and R^{13} are independently hydrogen or C_{1-6} alkyl, $CONR^{12}R^{13}$, or optionally substituted by C_{1-6}alkylR^9, aryl, phenoxy, COaryl, COheteroaryl or a heteroaryl group, the latter six groups being optionally substituted by halogen, amino, hydroxy, cyano, nitro, carboxy, $CONR^{12}R^{13}$, $SO_2NR^{12}R^{13}$, SO_2R^{12}, trifluoromethyl, $NHSO_2R^{12}$, $NHCOR^{12}$, ethylenedioxy, methylenedioxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkyl $NR^{10}R^{11}$, SR^{12} or $NR^{10}R^{11}$;

Het is a heteroaryl ring chosen from pyridine, pyrimidine, pyrazine, pyridazine or triazine and optionally substituted by halogen, amino, hydroxy, cyano, nitro, carboxy, $CONR^{12}R^{13}$, $SO_2NR^{12}R^{13}$, SO_2R^{12}, trifluoromethyl, $NHSO_2R^{12}$, $NHCOR^{12}$, C_{1-6} alkyl, C_{1-6} alkoxy, SR^{12} or $NR^{10}R^{11}$;

R^3 is independently hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^4 is independently hydrogen, C_{1-8} alkyl, C_{3-8} cycloalkyl, arylC_{1-5}alkyl or heteroarylC_{1-5}alkyl, the latter three groups being optionally substituted by one or more halogen, amino, hydroxy, C_{1-6} alkyl, C_{1-6} alkoxy, SR^{12} or $NR^{10}R^{11}$;

R^5 is independently hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^6 is independently hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^7 is independently hydrogen, C_{1-6} alkyl or C_{3-6} cycloalkyl;

R^8 is independently hydrogen, aryl, heteroaryl or C_{1-6} alkyl optionally substituted with one or more aryl, heteroaryl, halogen, amino, hydroxy, carboxy, $CONR^{12}R^{13}$, $SO_2NR^{12}R^{13}$, SO_2R^{12}, $NHSO_2R^{12}$, $NHCOR^{12}$, C_{1-6} alkyl, C_{3-6} cycloalkyl, C_{1-6} alkoxy, SR^{12} or $NR^{10}R^{11}$;

or a pharmaceutically acceptable salt thereof.

Aryl groups include phenyl and naphthyl. Heteroaryl groups include 5- or 6- membered, 5,6- or 6,6-fused heterocyclic rings containing one or more heteroatoms selected from N, S or O. Examples include pyridinyl, pyrimidinyl, thiazolyl, oxazolyl, pyrazole, imidazolyl, furyl, thienyl, quinolinyl, isoquinolinyl, benzimidazolyl, benzofuryl, benzothienyl and indolyl.

Aryl and heteroaryl groups can be optionally substituted by on or more of the following groups; halogen, amino, hydroxy, cyano, nitro, carboxy, $CONR^{12}R^{13}$, $SO_2NR^{12}R^{13}$, SO_2R^{12}, trifluoromethyl, $NHSO_2R^{12}$, $NHCOR^{12}$, ethylenedioxy, methylenedioxy, C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkyl $NR^{10}R^{11}$, SR^{12} or $NR^{10}R^{11}$.

Certain compounds of formula (I) are capable of existing in stereoisomeric forms. It will be understood that the invention encompasses all geometric and optical isomers of the compounds of formula (I) and mixtures thereof including racemates. Tautomers and mixtures thereof also form an aspect of the present invention.

Preferably R^1 is hydrogen or C_{1-6}alkyl, more preferably methyl and R^2 is CH_2R^9 or $CH_2CH_2R^9$ where R^9 is phenyl or a 5- or 6-membered aromatic ring containing one or two heteroatoms and optionally substituted by C_{1-6}alkyl. More preferably R^2 is CH_2R^9 or $CH_2CH_2R^9$ where R^9 is phenyl, pyridyl or oxazole substituted by methyl.

Alternatively R^1 and R^2 form a piperidine, piperazine, pyrrolidine, morpholine, or thiomorpholine ring optionally substituted by CH_2OH, CH_2CH_2OH, hydroxy, $CONH_2$, phenyl, phenoxy, C(O)-furyl, the latter three groups being optionally substituted by halogen, in particular chloro.

Preferably Het is pyrimidine ring.

Preferably R^3 is hydrogen.

Preferably R^4 is hydrogen.

Preferably R^5 is C_{1-6} alkyl, more preferably iso-butyl.

Preferably R^6 is hydrogen.

Preferably R^7 and R^8 are both hydrogen.

Preferred compounds of the invention include:

N~1~-[Cyano(2-methoxyphenyl)methyl]-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-leucinamide

N~1~-[Cyano(2-methoxyphenyl)methyl]-N~2~-(2-piperazin-1-ylpyrimidin-4-yl)-L-leucinamide,

N-[Cyano(2-methoxyphenyl)methyl]-N-(2-morpholin-4-ylpyrimidin-4-yl)-L-phenylalaninamide

N~1~-[Cyano(2-methoxyphenyl)methyl]-3-cyclohexyl-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-alaninamide

N-[2-(Benzylamino)pyrimidin-4-yl]-N-(cyanomethyl)-L-phenylalaninamide

N-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N-(cyanomethyl)-L-phenylalaninamide

N-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N-(cyanomethyl)-L-phenylalaninamide

N~2~-[2-(Benzylamino)pyrimidin-4-yl]-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

N~2~-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

N~2~-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

N~1~-(Cyanomethyl)-N~2~-(4-morpholin-4-ylpyrimidin-2-yl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-hydroxy-4-phenylpiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[methyl(pyridin-3-ylmethyl)amino]pyrimidin-4-yl}-L-leucinamide

N~2~-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

N~2~-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide,

N~2~-{2-[4-(5-Chloropyridin-2-yl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide,

N~1~-(Cyanomethyl)-N~2~-{2-[methyl(thien-3-ylmethyl)amino]pyrimidin-4-yl}-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-(2-thiomorpholin-4-ylpyrimidin-4-yl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenylpiperazin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[2-(hydroxymethyl)piperidin-1-yl]pyrimidin-4-yl}-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]pyrimidin-4-yl}-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-hydroxypiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[4-(2-furoyl)piperazin-1-yl]pyrimidin-4-yl}-L-

N~2~-{2-[3-(Aminocarbonyl)piperidin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[methyl(2-pyridin-2-ylethyl)amino]pyrimidin-4-yl}-L-leucinamide

N~2~-[2-(4-Benzylpiperidin-1-yl)pyrimidin-4-yl]-N~1~-(cyanomethyl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-pyridin-2-ylpiperazin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenylpiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-{2-[4-(2-hydroxyethyl)piperidin-1-yl]pyrimidin-4-yl}-L-leucinamide

N~2~-{2-[4-(3-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenoxypiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

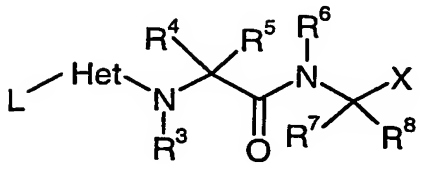
N~1~-(Cyanomethyl)-N~2~-[2-(3-phenylpyrrolidin-1-yl)pyrimidin-4-yl]-L-leucinamide

N~1~-(Cyanomethyl)-N~2~-(2-{methyl[(3-methylisoxazol-5-yl)methyl]amino}pyrimidin-4-yl)-L-leucinamide

and pharmaceutically acceptable salts thereof.

The present invention further provides a process for the preparation of a compound of formula (I) which comprises

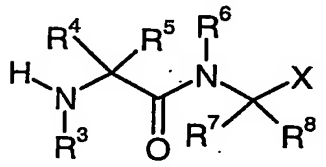
(i) reaction of a compound of general formula (II)



(II)

wherein L represents a leaving group (e.g. halide, sulphide, sulfoxide or sulphone group), preferably the sulphide is oxidised to a sulphoxide or sulphone group before displacement. An oxidising agent such as a peracid may be used, for example meta-chloroperbenzoic acid in dichloromethane at room temperature.
L may be displaced by NR^1R^2 respectively where R^1 and R^2 are defined in formula (I). The reaction may be performed in an inert solvent for example dioxane, N,N-dimethylformamide at ambient temperature or with heating, usually with a base present for example N,N-diisopropylethylamine.

X may be CN, or a group that can be readily converted into a nitrile, for example C1-6alkoxycarbonyl, $CONH_2$ or CO_2H.

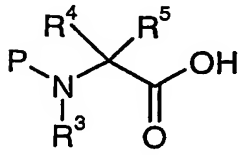


(III)

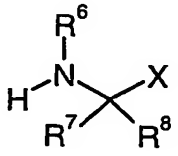
(IV)

Compounds of formula (II) may be prepared from compounds of formula (III) by displacement of a leaving group L^1 from compounds of formula (IV).
Wherein L^1 represents a leaving group (e.g. halide, sulphide, sulfoxide or sulphone group), preferably the sulphide is oxidised to a sulphoxide or sulphone group before displacement. An oxidising agent such as a peracid may be used, for example meta-chloroperbenzoic acid in dichloromethane at room temperature. The reaction may be performed in an inert solvent for example dioxane, N,N-dimethylformamide at ambient temperature or with heating, usually with a base present for example N,N-diisopropylethylamine.

Compounds of formula (III) may be prepared from the reaction of compounds of formula (V) with compounds of formula (VI) using an appropriate coupling agent , for example N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, carbonyl diimidazole. Alternatively the acid may be activated by formation of the acid chloride using for example, oxalyl chloride.



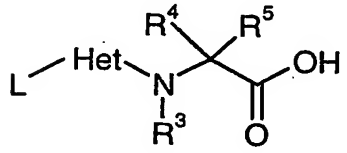
(V)



(VI)

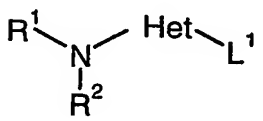
P is a nitrogen protecting group for example tert-butylcarbamate, benzyl carbamate, benzyl.

Compound of general formula (II) may also be prepared from the reaction of compounds of formula (VII) with compounds of formula (VI) using an appropriate coupling agent , for example N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, carbonyl diimidazole. Alternatively the acid may be activated by formation of the acid chloride using for example, oxalyl chloride.

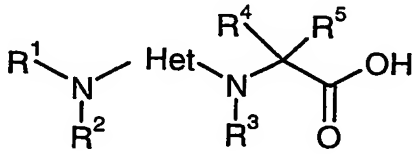


(VII)

(ii) reaction of a compound of general formula (VIII) with compounds of formula (III) or reaction of a compound of general formula (IX) with a compound of general formula (VI).



(VIII)



(IX)

According to a further feature of the invention there is provided a compound of the formula (I), or a pharmaceutically acceptable salt thereof, for use as a therapeutic agent.

According to a further feature of the present invention there is provided a method for producing inhibition of a cysteine protease in a warm blooded animal, such as man, in need of such treatment, which comprises administering to said animal an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof. In particular the compounds of the invention are useful in the treatment of inflammation and immune disorders such as, but not limited to, asthma, rheumatoid arthritis, COPD, multiple sclerosis, Crohn's disease, Alzheimers and pain, such as neuropathic pain. Preferably the compounds of the invention are used to treat pain, especially neuropathic pain.

The invention also provides a compound of the formula (I), or a pharmaceutically acceptable salt thereof, for use as a medicament; and the use of a compound of the formula (I) of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of a cysteine protease in a warm blooded animal, such as man.

In particular the invention provides the use of a compound of the formula (I) of the present invention, or a pharmaceutically acceptable salt thereof, in the manufacture of a medicament for use in the inhibition of Cathepsin S in a warm blooded animal, such as man. In order to use a compound of the formula (I) or a pharmaceutically acceptable salt thereof for the therapeutic treatment of mammals including humans, in particular in the inhibition of a cysteine protease, it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Therefore in another aspect the present invention provides a pharmaceutical composition which comprises a compound of the formula (I) or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable diluent or carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, rectal or parenteral administration. For these purposes the compounds of this invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions or suspensions, (lipid) emulsions, dispersible powders,

suppositories, ointments, creams, drops and sterile injectable aqueous or oily solutions or suspensions.

A suitable pharmaceutical composition of this invention is one suitable for oral administration in unit dosage form, for example a tablet or capsule which contains between 100 mg and 1 g of the compound of this invention.

In another aspect a pharmaceutical composition of the invention is one suitable for intravenous, subcutaneous or intramuscular injection.

Each patient may receive, for example, an intravenous, subcutaneous or intramuscular dose of 1 $mgkg^{-1}$ to 100 $mgkg^{-1}$ of the compound, preferably in the range of 5 $mgkg^{-1}$ to 20 $mgkg^{-1}$ of this invention, the composition being administered 1 to 4 times per day. The intravenous, subcutaneous and intramuscular dose may be given by means of a bolus injection. Alternatively the intravenous dose may be given by continuous infusion over a period of time. Alternatively each patient will receive a daily oral dose which is approximately equivalent to the daily parenteral dose, the composition being administered 1 to 4 times per day.

The following illustrate representative pharmaceutical dosage forms containing the compound of formula (I), or a pharmaceutically-acceptable salt thereof (hereafter compound X), for therapeutic or prophylactic use in humans:

(a)

Tablet I	mg/tablet
Compound X.	100
Lactose Ph.Eur.	179
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(b)

Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur.	229
Croscarmellose sodium	12.0
Polyvinylpyrrolidone	6
Magnesium stearate	3.0

(c)

Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur.	92
Croscarmellose sodium	4.0
Polyvinylpyrrolidone	2.0
Magnesium stearate	1.0

(d)

Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur.	389
Croscarmellose sodium	100
Magnesium stearate	1.

(e)

Injection I	(50 mg/ml)
Compound X	5.0% w/v
Isotonic aqueous solution	to 100%

Buffers, pharmaceutically-acceptable cosolvents such as polyethylene glycol, polypropylene glycol, glycerol or ethanol or complexing agents such as hydroxy-propyl β cyclodextrin may be used to aid formulation.

Note

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate.

The following examples illustrate the invention.

Example 1

N~1~-[Cyano(2-methoxyphenyl)methyl]-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-leucinamide

(i) N~2~-(tert-Butoxycarbonyl)-N~1~-[cyano(2-methoxyphenyl)methyl]-L-leucinamide

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.9g) and 1-hydroxybenzotriazole hydrate (2.0g) were added to a solution of 2-methoxyphenylamino acetonitrile (2.0g) and N-tert-butoxycarbonyl L-leucine (2.5g) in N,N-dimethylformamide (20ml) at room temperature followed by N,N-diisopropylethylamine (5.3ml) and stirred at room temperature overnight. The mixture was diluted with water, extracted into ethyl acetate and dried($MgSO_4$). The solvent was removed under vacuum to leave an oil which was subjected to column chromatography on silica eluting with isohexane/ethyl acetate 2:1 to give a colourless oil (3.7g).

MS: APCI(+ve) 249(M-Boc-CN+1)

(ii) N~1~-[Cyano(2-methoxyphenyl)methyl]-L-leucinamide

The product from step (i) (3.70g) in formic acid (40ml) was stirred for 90min at room temperature then the solvent was removed under vacuum to give a yellow oil (2.7g).

MS: APCI(+ve) 276(M-Boc+1)

(iii) N~1~-[Cyano(2-methoxyphenyl)methyl]-N~2~-(2-fluoropyrimidin-4-yl)-L-leucinamide

A solution of the product from step (ii) (2.7g) and N,N-diisopropylethylamine (1.7ml) in tetrahydrofuran (40ml) was added dropwise to a solution of 2,4-difluoropyrimidine (1.15g) in tetrahydrofuran (40ml) and N,N-diisopropylethylamine (1.7ml). After stirring at room temperature overnight the solvent was removed under vacuum to yield a crude oil which was subjected to column chromatography on silica eluting with dichloromethane/ethyl acetate 2:1 to give a colourless oil (1.50g).

MS: APCI(+ve) 372(M+1)

(iv) N~1~-[Cyano(2-methoxyphenyl)methyl]-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-leucinamide

The product from step (iii) (0.5g), morpholine (0.12ml) and N,N-diisopropylethylamine (0.24ml) in tetrahydrofuran (20ml) was stirred at room temperature overnight. The solvent was removed under vacuum to yield a crude oil which was subjected to column chromatography on silica eluting with ethyl acetate/isohexane 3:1 to give a white solid (0.4g).

MS: APCI(+ve) 439(M+1)
1H NMR: δ (DMSO) 9.40 (1H, m), 9.08 (1H, m), 7.78-7.12 (5H, m), 6.10-6.08 (1H, d), 5.80 (1H, m), 4.60-4.40 (1H, m), 3.84-3.51 (11H, m), 1.80-1.20(3H, m), 0.96-0.84 (6H, m).

Example 2
N~1~~-[Cyano(2-methoxyphenyl)methyl]-N~2~~-(2-piperazin-1-ylpyrimidin-4-yl)-L-leucinamide, trifluoroacetate salt

The title compound was prepared according to the procedure in example 1 step (iv) using piperazine.

MS: APCI(+ve) 438(M+1)
1H NMR: δ (DMSO) 8.83-8.81 (2H, m), 7.79-6.97 (5H, m), 6.09-6.02 (2H, m), 4.40 (1H, m), 3.85 (7H, bm), 3.13-3.05 (4H, m), 1.68-1.49 (3H, m), 0.94-0.84 (6H, m).

Example 3
N-[Cyano(2-methoxyphenyl)methyl]-N-(2-morpholin-4-ylpyrimidin-4-yl)-L-phenylalaninamide

(i) N-(tert-Butoxycarbonyl)-N-[cyano(2-methoxyphenyl)methyl]-L-phenylalaninamide
The sub-title compound was prepared from N-butoxycarbonyl-L-phenylalanine (1.32g) by the method of example 1 step (i). Yield 2.05g.

MS: APCI(+ve) 310 (M-Boc+1)

(ii) N-[Cyano(2-methoxyphenyl)methyl]-N-(2-fluoropyrimidin-4-yl)-L-phenylalaninamide

The sub-title compound was prepared from the product of step (i) (2.05g) by the method of example 1 steps (ii) and (iii). Yield 0.57g.

MS: APCI(+ve) 406 (M+1)

(iii) N-[Cyano(2-methoxyphenyl)methyl]-N-(2-morpholin-4-ylpyrimidin-4-yl)-L-phenylalaninamide

The title compound was prepared from the product of step (ii)(0.25g) by the method of example 1 step (iv). Yield 0.078g.

MS: APCI(+ve) 473 (M+1)
NMR: δ (DMSO) 9.29 and 9.15 (1H, 2xd), 7.73 and 7.69 (1H, 2xd), 7.45-7.40 (2H,m), 7.33-7.17 (6H,m), 7.11 (1H,m), 7.00 (1H,m), 6.08 (1H,dd), 5.88 and 5.85 (1H,2xd), 4.64 (1H, brs), 3.83 and 3.80 (3H, 2xs), 3.58 (4H,m), 3.47 (4H,m), 3.05-2.82 (2H,m).

Example 4
N~1~-[Cyano(2-methoxyphenyl)methyl]-3-cyclohexyl-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-alaninamide

(i) N-(tert-Butoxycarbonyl)-N-[cyano(2-methoxyphenyl)methyl]-3-cyclohexyl-L-alaninamide
The sub-title compound was prepared from N-butoxycarbonyl-beta-cyclohexyl-L-alanine (1.36g) by the method of example 1 step (i). Yield 1.99g. Used directly in the next step.

(ii) N~1~-[Cyano(2-methoxyphenyl)methyl]-3-cyclohexyl-N~2~-(2-fluoropyrimidin-4-yl)-L-alaninamide
The sub-title compound was prepared from the product of step (i) (1.99g) by the method of example 1 steps (ii) and (iii). Yield 0.12g.

MS: APCI(+ve) 412 (M+1)

(iii) N~1~-[Cyano(2-methoxyphenyl)methyl]-3-cyclohexyl-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-alaninamide

The title compound was prepared from the product of step (ii) (0.12g) by the method of example 1 step (iv). Yield 0.087g.

MS: APCI(+ve) 479 (M+1)

NMR: δ (DMSO) 9.18 and 9.06 (1H,2xd), 7.76 and 7.72 (1H,2xd), 7.49-7.37 (2H,m), 7.24 (1H,brs), 7.11 (1H,d), 7.02 (1H,t), 6.09 (1H,m), 5.91 and 5.88 (1H,2xd), 4.46 and 4.36 (1H,2xbrs,), 3.82 and 3.80 (3H,2xs), 3.60 (4H,m), 3.47 (4H,m), 1.76-1.36 (8H,m), 1.24-1.09 (3H,m), 0.98-0.83 (2H,m).

Example 5

N-[2-(Benzylamino)pyrimidin-4-yl]-N-(cyanomethyl)-L-phenylalaninamide

(i) N-(tert-Butoxycarbonyl)-N-(cyanomethyl)-L-phenylalaninamide

The sub-title compound was prepared from aminoacetonitrile hydrochloride by the method of example 1 step (i).

MS: APCI(+ve) 204 (M-Boc+1)

(ii) N-(Cyanomethyl)-N-(2-fluoropyrimidin-4-yl)-L-phenylalaninamide

The sub-title compound was prepared from the product of step (i) (3.5g) by the method of example 1 steps (ii) and (iii). Yield 1.11g.

MS: APCI(+ve) 300 (M+1)

(iii) N-[2-(Benzylamino)pyrimidin-4-yl]-N-(cyanomethyl)-L-phenylalaninamide

The title compound was prepared from the product from step (ii) (0.2g) and benzylamine (0.37ml) by the method of example 1 step (iv). Yield 0.11g.

MS: APCI(+ve) 387 (M+1)

NMR: δ (DMSO) 8.60 (1H,brs), 7.61 (1H,d), 7.29-7.14 (10H,m), 6.93 (1H,brs), 5.78 (1H,d), 4.64 (1H,brs), 4.47-4.33 (2H,m), 4.05 (2H,brs), 3.03 (1H,dd), 2.85 (1H,m).

Example 6

N-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N-(cyanomethyl)-L-phenylalaninamide

The title compound was prepared from the product of example 5 step (ii) (0.2g) and N-benzylmethylamine by the method of example 1 step (iv). Yield 0.18g.

MS: APCI(+ve) 401 (M+1)
NMR: δ (DMSO) 8.69 (1H,brs), 7.71 (1H,d), 7.33-7.15 (10H,m), 5.84 (1H,d), 4.75 (2H,q), 4.62 (1H,brs), 4.03 (2H,brs), 2.99 (1H,dd), 2.94 (3H,s), 2.86 (1H,m).

Example 7

N-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N-(cyanomethyl)-L-phenylalaninamide

The title compound was prepared from the product of example 5 step (ii) (0.2g) and 4(4-chlorophenyl)piperazine by the method of example 1 step (iv). Yield 0.18g.

MS: APCI(+ve) 476 (M+1)
NMR: δ (DMSO) 8.77 (1H,t), 7.72 (1H,d), 7.40 (1H,brs), 7.31-7.17 (7H,m), 6.98 (2H,d), 5.86 (1H,d), 4.54 (1H,brs), 4.13 (2H,m), 3.74 (4H,m), 3.12 (4H,m), 3.01 (1H,dd), 2.89 (1H,m).

Example 8

N~2~-[2-(Benzylamino)pyrimidin-4-yl]-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

(i) N-(tert-butoxycarbonyl)-N-(cyanomethyl)-3-cyclohexyl-L-alaninamide

The sub-title compound was prepared from N-butoxycarbonyl-beta-cyclohexyl-L-alanine (5.0g) and aminoacetonitrile hydrochloride (1.71g) by the method of example 1 step (i). Yield 4.09g.

MS: APCI(+ve) 210 (M-Boc+H)

(ii) N~1~-(Cyanomethyl)-3-cyclohexyl-N~2~-(2-fluoropyrimidin-4-yl)-L-alaninamide

The sub-title compound was prepared from the product of step (i) (4.09g) by the method of example 1 steps (ii) and (iii). Yield 1.00g.

MS: APCI(+ve) 306 (M+1)

(iii) N~2~-[2-(Benzylamino)pyrimidin-4-yl]-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

The title compound was prepared from the product of step (ii) (0.2g) by the method of example 1 step (iv). Yield 0.05g.

MS: APCI(+ve) 393 (M+1)

NMR: δ (DMSO) 8.48 (1H,brs), 7.64 (1H,d), 7.31-7.24 (4H,m), 7.17 (1H,m), 7.09 (1H,brs), 6.93 (1H,brs), 5.81 (1H,d), 4.47-4.36 (3H,m), 4.04 (2H,d), 1.75-1.47 (7H,m), 1.31 (1H,m), 1.19-1.09 (3H,m), 0.86 (2H,m).

Example 9

N~2~-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

The title compound was prepared from the product of example 8 step (ii) (0.2g) and N-benzylmethylamine (0.43ml) by the method of example 1 step (iv). Yield 0.13g.

MS: APCI(+ve) 407 (M+1)

NMR: δ (DMSO) 8.57 (1H,brs), 7.73 (1H,d), 7.31-7.27 (2H,m), 7.23-7.19 (4H,m), 5.85 (1H,d), 4.80 (2H,m), 4.42 (1H,brs), 4.02 (2H,m), 2.95 (3H,s), 1.69-1.44 (7H,m), 1.35 (1H,m), 1.24-1.07 (3H,m), 0.92-0.81 (2H,m).

Example 10

N~2~-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-3-cyclohexyl-L-alaninamide

The title compound was prepared from the product of example 8 step (ii) (0.2g) and 4(4-chlorophenyl)piperazine (0.66g) by the method of example 1 step (iv). Yield 0.2g.

MS: APCI(+ve) 482 (M+1)

NMR: δ (DMSO) 8.66 (1H,t), 7.75 (1H,d), 7.25 (3H,d), 6.98 (2H,d), 5.89 (1H,d), 4.35 (1H,brs), 4.12 (2H,d), 3.75 (4H,m), 3.13 (4H,m), 1.73-1.46 (7H,m), 1.37 (1H,m), 1.24-1.07 (3H,m), 0.97-0.87 (2H,m).

Example 11

N~1~-(Cyanomethyl)-N~2~-(4-morpholin-4-ylpyrimidin-2-yl)-L-leucinamide

(i) N~2~-(tert-Butoxycarbonyl)-N~1~-(cyanomethyl)-L-leucinamide

The sub-title compound was prepared according to the procedure of example 1 step (i) with amino acetonitrile hydrochloride (2.22g) and N-tert-butoxy S-leucine (5g).

MS: APCI(+ve) 270(M+1)

(ii) N~1~-(Cyanomethyl)-N~2~-(4-fluoropyrimidin-2-yl)-L-leucinamide and N~1~-(Cyanomethyl)-N~2~-(2-fluoropyrimidin-4-yl)-L-leucinamide

The sub-title compounds were prepared from the product of step (i) (4.3g) according to the procedure of example 1 steps (ii) and (iii).

N~1~-(Cyanomethyl)-N~2~-(4-fluoropyrimidin-2-yl)-L-leucinamide
Yield 0.38g
MS: APCI(+ve) 266(M+1)

N~1~-(Cyanomethyl)-N~2~-(2-fluoropyrimidin-4-yl)-L-leucinamide
Yield 3.8g
MS: APCI(+ve) 266(M+1)

(iii) N~1~-(Cyanomethyl)-N~2~-(4-morpholin-4-ylpyrimidin-2-yl)-L-leucinamide

The title compound was prepared according to the procedure of example 1 step (iv) using N~1~-(cyanomethyl)-N~2~-(4-fluoropyrimidin-2-yl)-L-leucinamide. Yield 0.2g

MS: APCI(+ve) 333(M+1)
1H NMR: δ (DMSO) 8.49-8.46 (1H, t), 7.83-7.81 (1H, d), 6.63 (1H, bm), 6.06-6.04 (1H, d), 4.25-4.05 (3H, m), 3.63-3.47 (8H, m), 1.75-1.39 (3H, m), 0.90-0.84 (6H, m).

Example 12

N~1~-(Cyanomethyl)-N~2~-(2-morpholin-4-ylpyrimidin-4-yl)-L-leucinamide

The title compound was prepared from N~1~-(cyanomethyl)-N~2~-(2-fluoropyrimidin-4-yl)-L-leucinamide (example 11 step (iii)) according to the procedure of example 1 step (iv). Yield 0.17g

MS: APCI(+ve) 333(M+1)
1H NMR: δ (DMSO) 8.64-8.60 (1H, t), 7.74-7.72 (1H, d), 7.24-7.23 (1H, d), 5.89-5.82 (1H, d), 4.31-4.08 (3H, m), 3.58 (8H, m), 1.72-1.39 (3H, m), 0.92-0.84 (6H, m).

Examples 13-34 were prepared according to the procedures of example 1 step (iv) using N~1~-(cyanomethyl)-N~2~-(2-fluoropyrimidin-4-yl)-L-leucinamide (example 11 step (iii)) and the appropriate amine.

Example 13
N~1~-(Cyanomethyl)-N~2~-[2-(4-hydroxy-4-phenylpiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 423(M+1)
1H NMR: δ (DMSO) 8.65-8.61 (1H, t), 7.73-7.14 (7H, m), 5.84-5.82 (1H, d), 5.00-4.39 (4H, m), 4.08-4.03 (2H, m), 3.20-3.12 (2H, m), 1.90-1.35 (7H, m), 0.92-0.85 (6H, m).

Example 14
N~1~-(Cyanomethyl)-N~2~-{2-[methyl(pyridin-3-ylmethyl)amino]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 368(M+1)
1H NMR: δ (DMSO) 8.59-7.20 (7H, m), 5.89-5.87 (1H, d), 4.68 & 4.37 (3H, m), 4.08-4.02 (2H, m), 2.99 (3H, s), 1.68-1.35 (3H, m), 0.93-0.80 (6H, m).

Example 15
N~2~-{2-[Benzyl(methyl)amino]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

MS: APCI(+ve) 367(M+1)
1H NMR: δ (DMSO) 8.57-8.54 (1H, t), 7.74 (1H, d), 7.31-7.18 (6H, m), 5.87-5.85 (1H, d), 4.82-4.00 (5H, m), 2.95 (3H, s), 1.71-1.40 (3H, m), 0.89-0.81 (6H, m).

Example 16

N~2~-{2-[4-(4-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide, trifluoroacetate salt

MS: APCI(+ve) 442(M+1)

1H NMR: δ (DMSO) 9.02-9.01 (2H, m), 7.75-6.98 (5H, m), 6.24-6.22 (1H, d), 4.48-4.13 (3H, m), 3.82-3.55 (8H, m), 1.66-1.50 (3H, m), 0.95-0.88 (6H, m).

Example 17

N~2~-{2-[4-(5-Chloropyridin-2-yl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide, bis trifluoroacetate salt

MS: APCI(+ve) 443(M+1)

1H NMR: δ (DMSO) 9.03-9.01 (2H, m), 8.15-6.90 (4H, m), 6.25-6.23 (1H, d), 4.49 (1H, m), 4.23-4.18 (2H, d), 3.80-3.66 (8H, m), 1.66-1.51 (3H, m), 0.95-0.88 (6H, m).

Example 18

N~1~-(Cyanomethyl)-N~2~-{2-[methyl(thien-3-ylmethyl)amino]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 373(M+1)

Example 19

N~1~-(Cyanomethyl)-N~2~-(2-thiomorpholin-4-ylpyrimidin-4-yl)-L-leucinamide

MS: APCI(+ve) 349(M+1)

Example 20

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenylpiperazin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 408(M+1)

Example 21

N~1~-(Cyanomethyl)-N~2~-{2-[2-(hydroxymethyl)piperidin-1-yl]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 361(M+1)

Example 22
N~1~-(Cyanomethyl)-N~2~-{2-[(2R)-2-(hydroxymethyl)pyrrolidin-1-yl]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 347(M+1)

Example 23
N~1~-(Cyanomethyl)-N~2~-[2-(4-hydroxypiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 347(M+1)

Example 24
N~1~-(Cyanomethyl)-N~2~-{2-[4-(2-furoyl)piperazin-1-yl]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 426(M+1)

Example 25
N~2~-{2-[3-(Aminocarbonyl)piperidin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

MS: APCI(+ve) 374(M+1)

Example 26
N~1~-(Cyanomethyl)-N~2~-{2-[methyl(2-pyridin-2-ylethyl)amino]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 382(M+1)

Example 27

N~2~-[2-(4-Benzylpiperidin-1-yl)pyrimidin-4-yl]-N~1~-(cyanomethyl)-L-leucinamide

MS: APCI(+ve) 421(M+1)

Example 28

N~1~-(Cyanomethyl)-N~2~-[2-(4-pyridin-2-ylpiperazin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 409(M+1)

Example 29

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenylpiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 407(M+1)

Example 30

N~1~-(Cyanomethyl)-N~2~-{2-[4-(2-hydroxyethyl)piperidin-1-yl]pyrimidin-4-yl}-L-leucinamide

MS: APCI(+ve) 375(M+1)

Example 31

N~2~-{2-[4-(3-Chlorophenyl)piperazin-1-yl]pyrimidin-4-yl}-N~1~-(cyanomethyl)-L-leucinamide

MS: APCI(+ve) 442/4(M+1)

Example 32

N~1~-(Cyanomethyl)-N~2~-[2-(4-phenoxypiperidin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 423(M+1)

Example 33

N~1~~-(Cyanomethyl)-N~2~~-[2-(3-phenylpyrrolidin-1-yl)pyrimidin-4-yl]-L-leucinamide

MS: APCI(+ve) 393(M+1)

Example 34
N~1~~-(Cyanomethyl)-N~2~~-(2-{methyl[(3-methylisoxazol-5-yl)methyl]amino} pyrimidin-4-yl)-L-leucinamide

MS: APCI(+ve) 372(M+1)

Measurement of Cathepsin S activity.

QFRET Technology (Quenched Fluorescent Resonance Energy Transfer) was used to measure the inhibition by test compounds of Cathepsin S-mediated cleavage of the synthetic peptide Z-Val-Val-Arg-AMC. Compounds were screened at five concentrations in duplicate and the pIC_{50} values reported.

Synthetic substrate, 20□M [final]Z-Val-Val-Arg-AMC in phosphate buffer were added to a 96 well black Optiplate. The assay plates were pre-read for compound auto fluorescence on SpectraMax Gemini at 355nM excitation and 460nM emission. 250pM [final] rHuman Cathepsin S in phosphate buffer was added and incubated for 2h at room temperature on the SpectraMax Gemini, taking readings every 20min at 355nM excitation and 460nM emission.

Activity Based template (5PTB-8) used the auto fluorescent corrected data to calculate the percentage inhibition for each compound concentration using the relevent plate controls. This data was used to construct inhibition curves and pIC_{50} estimated by non-linear regression using a 4 parameter logistic model.